IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of :

Ahrens-Fath, et al. Group Art Unit: 1645

Serial No.: 09/997,267 Examiner: TBA

Filed: November 30, 2001

For: NEW HUMAN ANDROGEN RECEPTOR VARIANTS

SUPPLEMENTAL PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

IN THE SPECIFICATION:

On page 20, replace the paragraph beginning on line 10 and continuing onto page 21, line 22 with the following amend paragraph:

Starting material was 1 µg of total-RNA from human placenta, which was converted by means of the SMART RACE amplification kit (Clontech) into cDNA. For PCR amplification, the Advantage-2 PCR kit (Clontech) was used together with an antisense primer (5'-CAGATTACCAAGCTTCAGCTTCCG-3'), (SEQ ID NO: 7) which is directed against the Hinge region of the human androgen receptor and uses a sense 5'-Smart II primer. The reaction conditions were: 5 seconds at 94°C, 3 minutes at 72°C (5 cycles); 5 seconds at 94°C, 10 seconds at 70°C, 3 minutes at 72°C (5 cycles); 5 seconds at 94°C, 10 seconds at 68°C, 3 minutes at 72°C (27 cycles). To this end, a fragment of about 500 base pairs was amplified, purified on agarose gel, cloned in the PCR-TOPO plasmid (Invitrogen) and sequenced. The DNA sequence showed that the complete DNA-binding domain of the androgen receptor was present (corresponds to Exons 2 and 3 in the androgen receptor gene). In addition, a new sequence was linked immediately before Exon 2. This section, which can be designated as Exon 1B, contains about 160 base pairs of the untranslated range

and a short, new sequence that codes for 7 amino acids. To isolate the complete cDNA, the sense primer 5'-ACAGGGAACCAGGGAAACGAATGCAGAGTGCTCCTGACATTGCCTGT-3' (SEQ ID NO: 8) (final concentration 0.2 µm) and 5'-GGACAGGGAACCAGGGAAACGAATG-3' (SEQ ID NO: 9) (final concentration 1 µm), which originate from the new Exon 1B-range, and an antisense primer (5'-TCACTGGGTGTGGAAATAGATGGGCTTGA-3') (SEQ ID NO: 10), which codes for the C-terminal end of the known AR, were synthesized. The specified conditions for the SMART-PCR were used. It thus is possible to amplify and to clone a fragment of about 1200 base pairs from the same cDNA placenta. After DNA sequencing it turned out that there were two different fragments, as shown in SEQ ID NO 1 and NO 3. In both cases, the new portion that corresponds to Example 1B was present. The difference between AR42 and AR32 was in the C-terminal range, since in AR32, the region that is coded by Exon 7 was missing. A search in the genomic data bases showed that the new Exon 1B range is in the middle of the first Intron of the androgen receptor gene. An analysis of the gene section that precedes it shows that a second promoter of the androgen receptor gene is possibly in this region. This section contains putative initiator regions that are used in the detection by the basal transcription machinery, as well as several putative steroid hormone-responsive elements.

On page 21, replace the paragraph beginning on line 25 and continuing onto page 22, line 16 with the following amend paragraph:

The tissue distribution was determined by semi-quantitative PCR. The primers, which were used for the isolation of complete AR42 and AR32-cDNA sequences (Example 1), were also used here. In the control, specific primers for beta-actin were used (sense primer: 5'-TGACGGGGTCACCCACACTGTGCCCATCTA-3' (SEQ ID NO: 11); antisense primer: 5'-CTAGAAGCATTTGCGGTGGACGATGGAGGG-3') (SEQ ID NO: 12). Total-RNA from the following human tissues was used: brain, testicle, kidney, liver, uterus, prostate, lung, trachea, muscle, breast, heart. After transcription in first-strand cDNA (Stratagene), a PCR analysis was performed with the Advantage-2 PCR kit (Clontech). The reaction conditions were: 5 seconds at 94°C, 3 minutes at 72°C (5 cycles); 5 seconds at 94°C, 10 seconds at 70°C, 3 minutes at 72°C (5 cycles); 5 seconds at 94°C, 10 seconds at 68°C, 3 minutes at 72°C (20 cycles). The amplification

products were separated on a 1% agarose gel and stained with ethidium bromide. The results showed that AR42 RNA was expressed most often in the heart, muscle, uterus and in the prostate. The AR32 RNA amounts were generally low and did not show any significant differences between tissues.

On page 24, replace the paragraph beginning on line 21and continuing onto page 25, line 4 with the following amend paragraph:

The expression of AR42 and AR32 mRNA in prostate tumors was determined by semi-quantitative PCR. The primers described in Example 2 were used. In the control, specific primers were used for human ribosomal protein S9 (sense primer: 5' - GATGAGAAGGACCCACGGCGTCTGTTCG-3' (SEQ ID NO: 13); antisense primer: 5' – GAGACAATCCAGCAGCCCAGGAGGGACA-3') (SEQ ID NO: 14) and for AR (sense primer: 5'-CCCTGGATGGATAGCTACTCCGGACCTTACGGGGACATGCGT-3' (SEQ ID NO: 15); antisense primer: 5'-TCACTGGGTGTGGAAATAGATGGGCTTGA-3') (SEQ ID NO: 10). Whole RNA from normal prostate tissue or from prostate tumors was analyzed as in Example 2. The optical density of the bands was measured with the Gel Doc system and the Quantity One software of Biorad.

IN THE CLAIMS:

Please amend claim 10 as follows:

10. (Twice Amended) Use of an antibody for detection of a polypeptide coded by a nucleic acid of claim 1 in the tumor tissue.

REMARKS

The amendments to the specification have been made to properly identify the sequence listings.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

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Attorney Docket No.: SCH-1793

Date: June 6, 2002

VERSION WITH MARKINGS TO SHOW CHANGES MADE

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antisense primer: 5'-TCACTGGGTGTGGAAATAGATGGGCTTGA-3') (SEQ ID NO: 10). Whole RNA from normal prostate tissue or from prostate tumors was analyzed as in Example 2. The optical density of the bands was measured with the Gel Doc system and the Quantity One software of Biorad.

IN THE CLAIMS:

Please amend claim 10 as follows:

10. (Twice Amended) Use of an antibody [according to claim 9] for detection of a polypeptide coded by a nucleic acid of claim 1 in the tumor tissue.